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<b>(54) Title:</b> ANTIVIRAL COMPOSITIONS AND METHODS		
<b>(57) Abstract</b>		
<p>Antiviral compositions and methods are described. A preferred composition is an emulsified oil adapted for application to the entire epidermal surface of a person, and containing an active ingredient which is preferably an antiviral proteolytic enzyme, and optionally an enzyme synergist, both extracted from or replicating components of the saliva of a blood-feeding insect.</p>		

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ANTIVIRAL COMPOSITIONS AND METHODS

This invention relates to compositions and methods for the treatment or prevention of disease in humans and  
5 animals.

There is general concern that viral, microbial, bacterial, cancer and protozoan diseases are on the increase. Of particular concern are infections such as  
10 encephalitis, HIV, malaria, trypanosomiasis, ameobiasis, haemorrhagic fever, ebola, tuberculosis, brucellosis, systic lupus, erythematosus, Crohn's disease, meningitis, dengue, yellow fever, filariasis, Rift Valley fever, skin cancer, hepatitis, swamp fever, typhoid, rubella, herpes,  
15 viral meningitis and influenza.

Considerable research effort has been and is currently focused on the identification of curative treatments for viral, microbial, bacterial, cancer and  
20 protozoan diseases. While some advanced drugs have been found to be effective against several specific infections, only limited progress has been made against the broad spectrum of such diseases. There is particular concern about the ineffectiveness of existing pharmaceutical  
25 compositions and methods in relation to diseases causes by viruses.

In many cases the development of recognisable symptoms of viral, microbial, bacterial, cancer and  
30 protozoan diseases occur long after the infection and are indicative of advanced stages of infection. At such advanced stages these diseases, especially those diseases caused by viruses, may be untreatable and are frequently terminal.

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Most of the above listed infections are caused by penetration of a human epidermal or mucosal surface by infective agents. Many tropical diseases, for example, are caused by penetration of the skin by blood-feeding insects such as mosquitoes, e.g. Anopheles or Aedes, or the Ceratopogonidae family i.e. midges. Such penetrations by insects carry infective agents into the lymphatic or blood systems of humans, causing many of the above listed diseases. Other diseases may be transmitted as a consequence of infective agents alighting on epidermal or mucosal surfaces.

It has been observed that specific insects and even specific genera within families of insects, transmit specific diseases to man and/or animals. Conversely, I believe that some genera within these same families of insects exhibit refractoriness towards infective agents. This is to say that specific genera within these families of insects may possess chemical mechanisms that may destroy, nullify or inactivate specific infective agents.

These specific genera of insects that I believe exhibit specific refractoriness penetrate human and/or animal skin with their proboscis, which is lubricated, moistened, wetted or filled with saliva. This saliva contains enzymes to initiate pre-digestion of the insect's chosen food, which may, for example, be human blood.

My insight which underpins certain aspects of the present invention is that the saliva of specific insects which exhibit specific refractoriness towards specific human and/or animal diseases contains chemically active ingredients that destroy, nullify or inactivate specific infective agents, including viruses.

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The pharmacological properties of the salivas of certain organisms have been investigated, and have given rise to prior inventions.

5 In relation to leeches, PCT/NL90/00046 describes a novel inhibitor having anti-elastase and anti-chymotrypsin activity, derived from the "Buffalo" leech species *Hirudinaria manillensis*. U.S. 4588587 describes a leech salivary gland extract having an anticoagulant effect and  
10 protease inhibitory effect, with the potential to inhibit metastasis of malignant cells. WO 85/04418 relates to techniques for producing the leech anticoagulant hirudine by cloning techniques. U.S. 5246715 describes a composition with potential as an inhibitor of blood  
15 platelet aggregation, the composition being derived from leech salivas and having several low molecular weight components.

G.B. 881530 describes a substance derivable from  
20 animal or cattle saliva, able to accelerate the calcification of incisor dentine.

U.S. 5093322 describes a protein derivable from hard ticks, and having anticoagulant properties.  
25

WO 93/05150 and WO 94/13807 describe a natural or reproducible protein which can be isolated from a blood-sucking bug *Triatoma pallidipennis*, and which has anti-clotting and/or anti-cancer properties.

30 In accordance with a first aspect of the invention, there is provided a composition for the antiviral treatment of a human or animal, the composition containing one or more active antiviral agents based on or extracted  
35 from the saliva of a blood-feeding arthropod, or

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replicating in whole or in part one or more component(s) of such saliva.

The arthropod may for example be a blood-feeding tick (Acarina) or insect (Insecta). Suitably it is an insect. Preferably it is an insect of the family Ceratopogonidae (midges) or, especially, of the family Culicidae (mosquitos). For information about mosquitos and midges the reader is referred to Manson's Tropical Diseases, P.E.C. Manson, 18th edition. Bailliere Tind., 1982, Appendix III; and to the further articles and books referred to therein. Potentially any of the blood-feeding mosquitos or midges referred to therein could be of significance in relation to the present invention and the names of those are incorporated herein by reference. However, of particular interest, I believe, are the mosquito genera *Anopheles*, *Mansonia*, *Aedes* and *Culex*; of these *Anopheles* may be of particular significance, as may the *Aedes* subgenera *Aedes Stegomyia*, this subgenera including *Aedes aegypti*.

According to a second aspect of the present invention there is provided a composition for the antiviral treatment of a human or animal, comprising an enzyme as an active antiviral agent.

This aspect of the present invention is based on the insight that the saliva of blood-feeding insects must contain agents which exhibit antiviral properties against specific viruses, whilst clearly acting as vectors for the transmission of other specific viruses. Whilst detailed analysis of the saliva of appropriate insects has yet to be carried out and will take time and cost money, it is believed that, using state of the art techniques of extraction, separation, activity testing and analysis used

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in the chemical and biological spheres, it will be a routine if time-consuming task to elicit antiviral agents in the insect salivas, together with their structures and modes of action; and that, using state of the art techniques of synthetic chemistry, fermentation biology and molecular biology, including cloning, it will be a routine if time-consuming task to devise methods for replicating those antiviral agents synthetically. Such techniques of extraction, separation, activity testing, structural analysis, synthetic chemistry, fermentation biology and molecular biology are described in many textbooks and furthermore are exemplified in relation to precedent teachings described in many of the earlier patent specifications and journal articles referenced in this specification. To that extent that they describe such techniques which can be put to use in relation to the present invention, those patent specifications and journal articles are incorporated herein by reference.

By "antiviral" in this specification we mean any agent which renders a virus unable to infect a human or animal, or suppresses, reduces or redirects the activity of the virus. I believe that antiviral enzymes in the salivas of blood-feeding insects may be acting to suppress, reduce or redirect the activity of viruses, rather than destroying them.

Whilst this second aspect of the invention has arisen from considerations related to the refractoriness exhibited by blood-feeding insects it should be noted that in relation to this second aspect I claim a composition containing any enzyme, as an active antiviral agent. Experiments have shown that even broad spectrum industrial enzymes may exhibit such activity against poliomyelitis virus.

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I also believe that enzymes of utility in relation to the second aspect of the invention may show enhanced activity when used in conjunction with a synergist for the enzyme. By "synergist" I mean any agent which increases  
5 the activity of the enzyme against a target virus. Again, whilst this proposal arises from consideration of biological systems and is likely to apply to enzymes derived from saliva of blood-feeding insects, it is likely too that enzymes derived from other sources may have  
10 synergists, albeit that they may be more difficult to determine.

It is known that salival enzymes of organisms contain agents which inhibit the enzymes' activity against e.g.  
15 certain proteins. For example human saliva is known to contain a protein called secretory leucocyte protease inhibitor (SLPI) which is believed to inhibit the movement of HIV virus across a cell's outer membrane (McNeely et al, Jnl. of Clinical Investigation, vol. 96, July 1995,  
20 456-454). It is also clear that enzymes must be able to attack certain proteins e.g. found in meat or blood whilst not attacking very similar or identical proteins within the human body. Arising from this background I believe that routine if time-consuming work will show the  
25 existence of enzyme synergists; and that the enzyme/enzyme synergist combinations may offer a substantial advance in antiviral technology.

Mucins present in insect salivas may also be  
30 effective as antiviral agents or as synergists of antiviral enzymes. Mucins are large protein molecules on which viruses or virus fragments may agglomerate. A composition of the present invention may also contain a mucin which enhances antiviral activity.

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Relevant scientific work on the profiling of enzymes and on enzyme-activator/inhibitor combinations is reported in the following:

- 5           Enzymes in saliva from four parasitic arthropods.  
          Kerlin R.L. et al., Med. Vet. Entomol., (1992 Apr. 6)  
          (2) 121-6.
- 10           Characterisation of a vasodilator from the salivary  
          glands of the yellow fever mosquito *Aedes aegypti*.  
          Ribeiro J.M., J. Exp. Biol, (1992 Apr.) 165 61-71.
- 15           5-Hydroxytryptamine in the salivary glands of adult  
          female *Aedes aegypti* and its role in regulation of  
          salivation. Novak M.G., J. Exp. Biol. (1995 Jan.)  
          198 167-74.
- 20           The salivary gland-specific apyrase of the mosquito  
          *Aedes aegypti* is a member of the 5'-nucleotidase  
          family. Champagne D.E. et al., U.S.A., (1995 Jan.  
          31) 92 694-8.
- 25           Isolation and characterisation of the gene expressing  
          the major salivary gland protein of the female  
          mosquito, *Aedes aegypti*. James A.A. et al., Mol.  
          Biochem. Parasitol., (1991 Feb.) 44 (2) 245-253.
- 30           The mechanism by which such agents may be present in  
          salivas may destroy, nullify, inactivate, suppress or  
          redirect the activity of specific infective viral agents  
          is believed to be that synergists, for example secretory  
          leucocyte protease synergists, can modify the activity of  
          proteolytic enzymes so that they can only key onto  
          specific linkages in specific protein chains e.g. onto  
35           specific linkages in the protein chains of the external

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protein envelopes of specific viruses. These specific linkages characterise the protein chain of the infective virus. Once attached to these specific protein linkages the proteolytic enzyme breaks the chain in the protein at  
5 that point. This chain breakage may destroy, nullify, inactivate, suppress or redirect the infective agent. The role of mucins may be to entrap the broken-off protein chains, thereby preventing re-formation of the infective agents.

10

However, the mechanism by which the different aspects of the invention may work is not limiting, as to those aspects.

15 The composition as described above may contain active ingredients composed of saliva(s), salival extract(s), or derived or replicated or synthesised enzyme(s), optionally with synergist(s) e.g. of the secretory leucocyte protease type, and optionally with mucin(s). The source of such  
20 salivas or saliva extracts or saliva replicants will be blood-sucking arthropods, especially insects.

The active agent(s) in the saliva(s) may be isolated, validated by testing against target organisms, analysed  
25 and then synthesized, using the techniques of physical, analytical and organic chemistry, and following biological testing. Furthermore, the gene(s) responsible for the active agent(s) may be identified. Molecular biology offers the possibility of providing the gene(s) in  
30 organisms, to produce for example transgenic sheep or cattle, able to produce much larger quantities of saliva containing the active ingredient(s).

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More specifically, I believe that the following analysis may prove to be correct (without limitation to my patent protection, if it is not).

5        There is a specific enzyme manufactured in the  
salivary glands of specific blood-feeding insects by a  
specific cDNA clone. In *Aedes aegypti* (f.) this is a  
pharmacologically active substance of the apyrase class.  
It is known to be a member of the "ubiquitous" 5'-  
10    nucleotidase family. Its role, as far as the insect is  
concerned, is to control salivation. Pharmacologically it  
is a stimulant of immunoreactive innervation. Biochemically, this substance is 5-hydroxytryptamine, that  
is 5-HT serotonin. I believe that this substance may  
15    prove to be an enzyme having antiviral activity, or an  
enzyme synergist.

The cDNA clone designated D7 produces a protein that  
is specific to specific insects. In *Aedes aegypti* (f.) it  
20    is a protein of approximately 37 kDA and its function, as  
far as the insect is concerned, is to aid blood feeding  
from specific hosts. I believe this substance may be  
antiviral or may be a synergist of an antiviral enzyme.

25        There is present in the saliva of *Aedes aegypti* a  
vasodilatory peptide of the tachykinin family. This  
vasodilatory activity is endothelium dependent heat stable  
and sensitive to both trypsin and chymotrypsin treatments,  
and both smooth muscle activities cross-desensitise to the  
30    tachykinin peptide substance "P". Molecular sieving has  
shown the substance to have a molecular mass of 1400. I  
believe this substance may be antiviral or may be a  
synergist of an antiviral enzyme.

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Many viruses have a protein "shell" (capsid).  
Suitably said enzyme comprises a proteinase.

Some viruses have a outer carbohydrate layer in order  
5 to try and evade a biological defence mechanism. Suitably  
said enzyme comprises a hydroxylase.

A composition in accordance with this second aspect  
of the invention may suitably be a combination of  
10 antiviral agents, for example selected from a proteinase,  
a hydroxylase, a synergist for one or both of those, and  
a mucin. Thus, the composition may comprise a "cocktail"  
of antiviral agents able to combat a wide range of virus-  
vectored diseases.

15 Whilst an enzyme for use in a composition according  
to this second aspect may be derived from any source, it  
is preferably based on or extracted from the saliva of a  
blood-feeding arthropod, as further defined above in  
20 relation to the first aspect of the present invention.  
Thus, a preferred composition is in accordance with both  
the first and second aspects of the present invention.

A composition in accordance with the first and/or  
25 second aspect of the present invention may be for curative  
use, to halt the onset of a viral infection already  
present, or more preferably, may be for preventive  
(prophylactic) use.

30 Preferably, a said composition is for the treatment  
of humans against a viral infection. Most preferably it  
is for the prevention of infection of humans by a virus  
selected from a picornavirus, for example poliomyelitis or  
viral hepatitis type A, a togavirus, for example yellow

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fever, dengue or encephalitis, or a retrovirus, for example HIV, or a cancer-causing virus.

The composition may be administered to the human or  
5 animal in any way, for example orally, parenterally or, preferably, by external application. Preferably it is applied to an epidermal or mucosal surface, for example, rectally, orally or by application to skin. It is expected that the composition will provide a prophylactic  
10 effect by preventing or inhibiting the penetration of a mucosal or epidermal surface, by an infective agent. The nature of the infective agent may determine the mode of application. For example, when the composition is for the prevention of infection by the HIV virus as a result of  
15 sexual contact it may be applied as an oil or cream, for example to genital regions. Alternatively or additionally it may then be comprised in a formulation comprising a lubricating oil or a spermicidal cream.

20 In this specification what is meant by a mucosal surface is a "moist" secretory surface such as found in the oral, nasal, vaginal and anal cavities; and an epidermal surface is a "dry" surface - i.e. ordinary skin.

25 The composition may be comprised in a formulation with an antimicrobial agent (which may be an antibacterial and/or an antifungal agent), and/or a deodorant, and/or an antiperspirant, and/or sun-screening agent and/or a moisturizer, and/or an insect repellent, and/or a scent  
30 and/or an aromatherapy-style essential oil. Given that many infective agents are transmitted by insects a preferred further ingredient is an insect repellent, for example pyrethrum or N,N-diethyl-3-methyl benzamide (Trade Mark DEET). When the infective agent is known to be able  
35 to penetrate the body at any epidermal site the

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composition may be applied to substantially the entire epidermal surface of the patient. This may be achieved by means of an all-over body cream. However I have found that, surprisingly, a more effective epidermal application  
5 is achieved by formulating the composition as an emulsion, for example, an emulsified oil or wax, which can be applied to the human body by an aqueous delivery means, for example by means of an aqueous shower or bath.

10       Delivery from a shower may be achieved by provision of a modified shower unit. At the end of a shower, the user may trigger the unit to add to the shower water the emulsion - just as a metered dose e.g. of a fertiliser may be added when required to a horticultural water spray.  
15       Delivery from a bath, which is preferred, involves the user pouring an emulsion onto the bath water, the emulsion containing the active antiviral agent(s) and optionally one or more further ingredients as mentioned above, immersing him/herself in the water, and then climbing out.  
20       Drying could be by means of warm air but I have found that drying by means of a towel is quite adequate - the emulsion is normally a moisturiser attracted to and compatible with the skin and tends not to be removed to any substantial degree by the towel. Indeed, the  
25       towelling action may improve the coating. Suitably, the towel may be a disposable non-woven paper or fabric, optionally impregnated with a composition containing one or more active agent(s).

30       In extremely dangerous situations an undiluted emulsion may be applied to the epidermal surface, for example by immersion in an "oil bath" or application by means of a fabric or sponge.

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This third aspect of the invention is believed to be novel. The prior art of which I am presently aware in relation to compositions applied to the epidermal surface in order to prevent infection therethrough is as follows.

5

Several specifications relate to lubricatant/spermicidal/anti-HIV/antifungal/antibacterial compositions for genital application, namely EP-A-636374, EP-A-547294, EP-A-402078, WO 95/17165, WO 95/15157, WO 93/09793.

10

Several specifications relate to anti-herpes compositions, for genital and/or oral application, namely EP-A-135713 and US 4185097. WO 86/02267 relates primarily to anti-herpes treatment by administration of a sulfosuccinate, but also discloses activity against other viruses.

15

EP-A-395215 discloses a protective gel for coating skin surfaces, particularly the hands of surgical attendants prior to putting on surgical gloves. The gel is water repellent, and may contain nonoxynol-9 as an active anti-infective agent.

20

EP-A-243145 describes an anti-microbial composition especially for veterinary use, adapted to be applied to at-risk areas of the skin or mucous membrane, for example several times daily to prevent bacteria, fungi, mould or the like from forming on an animal's skin, teats, ears and eyes.

25

30

WO 94/15461 describes a protective cream to be applied to the skin to protect health care workers from infection. The cream dries to form a barrier and may contain an antimicrobial compound.

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WO 93/18745 describes an antiviral cream or lotion for application to mammalian skin, under a protection skin barrier such as a surgical glove or condom.

5 WO 92/16201 describes certain combinations of betaines and amine oxides, for inhibiting the activity of enveloped viruses for which a major mode of transmission is sexual. The combinations are also spermicidal. Suppositories, a contraceptive sponge, spermicidal gels,  
10 creams, jellies and a contraceptive film are described in the examples.

US 4939123 describes antibacterial compositions having active ingredients prepared enzymatically from  
15 vegetable flour. The compositions can be formulated for topical administration, for example for ophthalmological application.

US 4374126 describes a film forming antimicrobial  
20 material for animal skin. The material comprises an acrylate polymer, an antimicrobial agent, an adhesion promotion and a cross-linking agent.

Mucosal surfaces may also be protected by  
25 compositions in accordance with the invention, for example by means of spermicidal oils or creams used before sexual intercourse, slow-release suppositories, or creams applied, for example, within the nasal cavity. The oral cavity may be difficult to protect due to the salivatory  
30 action but a composition may be formulated to apply a reasonably long-lasting barrier layer to the throat.

In accordance with a third aspect of the present invention is provided a composition adapted for  
35 application to substantially the entire epidermal surface



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of the human body, the composition comprising an active antiviral agent and being such that after such application the active antiviral agent is present on substantially the entire epidermal surface of the human body for a  
5 substantial period.

By "substantial period" I mean that the active antiviral agent(s) is/are present on the surface in an amount sufficient to give an antiviral effect for at least  
10 4 hours, suitably at least 8 hours, and preferably for at least 12 hours. Most preferably I mean that the active antiviral agent(s) is/are present on the surface in an amount sufficient to give an antiviral effect for at least  
15 24 hours.

The composition used in the third aspect of the invention is preferably an emulsion. A first hydrophobic component may be an oil, wax or jelly. A second component is water. The first component may suitably be derived  
20 from an animal, vegetable or mineral source. Examples of animal sources are lanolin and beeswax. Examples of vegetable sources are flowers, nuts, seeds, leaves and rinds. An example of a mineral source is a crude oil. An oil may thus be a vegetable oil (fatty acid). A wax may  
25 be a fatty acid ester, for example derived from a vegetable source, or from bees or wool, or may be a paraffinic wax. The jelly may be a paraffinic jelly such as petroleum jelly. A vegetable oil or a wax is preferred. The emulsion comprises a major proportion of  
30 a said first component, compatible with the epidermal surface to which the composition is to be applied, a minor proportion of water, and a surface active agent. By "major proportion" I mean at least 55% by volume, preferably at least 78% by volume, most preferably 75-88%  
35 by volume; by "minor proportion" I mean up to 40% by

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volume, preferably up to 20% by volume, most preferably 10-20% by volume. The surface active agent is preferably present in an amount up to 5% by volume, preferably up to 2% by volume.

5

Suitable oils include vegetable oils which are non-toxic to human skin and which preferably have a moisturising effect. Examples include olive oil, grape seed oil, sunflower oil, safflower oil, macadamia oil, 10 coconut oil, jojoba oil and blends thereof.

Suitable waxes include paraffin waxes and, especially carnuba wax, beeswax and wool waxes.

15 Further ingredients may be present, for example glycerine and/or lecithin, in an amount up to 40% by volume of the total, preferably up to 20% by volume. In the event that there any such further ingredients the percentages by volume of the "core constituents" (the said 20 first component, water and the surface active agent) stated above are scaled down accordingly in proportion.

The surface active agent may be selected from one or more of the following: alkoxylates, for example 25 alkylphenol alkoxylates, alcohol alkoxylates, polyol alkoxylates, amine alkoxylates, ester alkoxylates and acid ethoxylates; sulphonates, for example alkylaryl sulphonates, alkane sulphonates; alkane sulphates, for example sodium lauryl sulphates; ether sulphates, for 30 example sodium lauryl ether sulphates; alkane phosphates; and esters of alkylene oxide polymers.

In accordance with a further aspect of the present invention there is provided a method for the antiviral 35 treatment of a human or animal, by use of any composition

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of the first, second or third aspects of the invention defined above.

Specific embodiments of the invention will now be  
5 described by way of example.

An antiviral barrier coating designed for use as a preventative measure against tropical diseases such as dengue, yellow fever, Rift Valley fever, swamp fever and  
10 typhoid, is based on blended or replicated salival extracts from the Mosquito generas *Aedes*, *Culex* and *Mansonia*. The salival extracts or replicants are believed to contain specific proteolytic enzymes plus specific secretory leucocyte protease inhibitors plus specific  
15 mucins, and can destroy, nullify, inactivate, suppress or redirect the target infective agents, in particular viruses. The salival extract or replicant is made up into an emulsion based on cyclomethicone, ethyl hexyl stearate, lauryl octoate, cetyl palmitate, glycerine, carnuba wax,  
20 sodium citrate and water. The barrier coating is contained in a cartridge which is loaded into a venturi valve of a shower unit so as to coat the whole body. At the end of the shower the venturi may be operated to apply the protective material which is allowed to dry. To  
25 assist this a hot air blower may be provided.

A barrier coating may be designed for use as a preventative measure against HIV infection in the event of possible contact with infected body fluids e.g. in  
30 invasive operations or other invasive clinical procedures. This barrier coating is based on blended or replicated salival extracts from the Mosquito genera *Anopheles*. The salival extract or replicant is made up into an emulsion based on sunflower oil, jojoba oil, carnuba wax, sodium  
35 lactate, lanolin, lecithin and a suitable surface active

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agent. This barrier coating is contained in conventional bottles for pouring onto the surface of a warm water bath, so as to coat the whole body.

5       An antiviral barrier coating may be designed for use also as a preventative measure against skin cancers. This barrier coating is based on blended or replicated salival extracts from *Aedes aegypti* mosquitoes. The salival extract or replicant is made up into a water based cream  
10 based on glycerine, macadamia oil, coconut oil, sub-micron zinc oxide and water. This barrier coating is contained in tubes for application to all exposed parts of the body e.g. when working outside or sunbathing.

15       Another barrier coating composition, designed for use in epidemic situations for combating ebola would be based on replicated or extracted mosquito salival extracts together with industrially produced enzymes, together with glycerin, lecithin, lanolin and beeswax, together with  
20 ethylhexyl stearate as an emulsifying agent. The composition is applied to the epidermal surface by complete immersion in an undiluted bath thereof.

A preferred composition for day to day use is an  
25 emulsified oil containing 40% by volume olive oil, 40% by volume sunflower oil, 18% by volume distilled water, together with selected antiviral agent(s), 2% by volume of a surfactant dodecylbenzene sulphonate, small amounts of the aromatherapy oils lavender oil and lemon oil, and of  
30 the insect repellent N,N-diethyl-3-methyl benzamide. This composition is poured onto tepid water in a bath, where it spreads out over the surface. The person immerses completely in the bath then steps out and, preferably, towels dry in the usual way, to give a pleasant  
35 moisturized feel on the skin, with the antiviral agents

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thereon. In this composition the selected antiviral agent(s) may be a cocktail of agents derived from mosquito salivas. Alternatively or additionally nonoxynol-9 may be used as an antiviral agent.

5

As mentioned previously routine if time-consuming work will be required in order to determine the antiviral agents in the salivas of blood-feeding insects, and, preferably, to determine their structure and replicate them. Whilst I have not yet been able to commission this work I have commissioned work, on a confidential basis, undertaken by the Institute of Virology and Environmental Microbiology, Oxford, U.K., on the testing of some crude industrial enzymes, against a poliovirus type 1. This was carried out as follows.

15

#### Methods

Four enzymes supplied by Biocatalysts Limited of Treforest Industrial Estate, Wales, were used in a TCID<sub>50</sub> assay for poliovirus type 1.

	Code	Product name	Batch no.	Sample	Concentration
25	A	Macer 8R	1070395	liquid	1mg/ml
	B	Promod 198	3954944	liquid	1mg/ml
	C	TP433/4	587396	powder	1mg/ml
	D	Promod 278	2966078	powder	1mg/ml

All infectivity assays were undertaken using Hela cells (obtained from the Sir William Dunn School of Pathology, Oxford) which are susceptible to polivirus infection. Infectivity assays were performed using both cell monolayers and cell suspensions in RPMI supplemented with 5% foetal bovine serum (RPMI-5). 10-fold serial

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- 20 -

dilutions of virus preparation ( $10^{-2}$  -  $10^{-9}$ ) were made in RPMI supplemented with 10% foetal bovine serum (RPMI-10). Each enzyme dilution (100  $\mu$ l) plus the virus dilution (100  $\mu$ l) was incubated for 1 hour at 37°C and either  
5 absorbed onto the cell monolayers of a 96 microtitre plate or incubated in the absence of cells. Following incubation the enzyme/virus mixtures were removed from the monolayers and the cells overlaid with 200  $\mu$ l of RPMI-10. Alternatively, 100  $\mu$ l of a  $2 \times 10^4$  cell suspension in RPMI-  
10 10 and 100  $\mu$ l of enzyme/virus was dispensed into a 96 well microtitre plate. Plates were incubated for 48 hours at 37°C in a CO<sub>2</sub> incubator prior to reading.

## Results

15

The results of the infectivity assays in both cell monolayers and cell suspension monolayers are shown in Figure 1 a-e and 2 a-e, respectively. A reduction of infectivity at a virus dilution of  $10^{-6}$  was observed in both  
20 cell monolayers and suspension monolayers with enzyme A and in cell monolayers with enzyme B. Further work is needed to establish the statistical significance of this interesting preliminary finding.

## 25 Figure Legend:

4+	100% cytopathic effect (CPE)
3+	75% CPE
2+	50% CPE
30 1+	25% CPE
-	little or no CPE

Figure 1A. Monolayer - control

-2	-3	-4	-5	-6	-7	-8	-9	Cell - control			
4+	4+	3+	3+	1+	1+	-	-	-	-	-	-
4+	4+	3+	3+	2+	1+	-	-	-	-	-	-
4+	4+	3+	3+	3+	1+	-	-	-	-	-	-
4+	4+	3+	3+	1+	1+	-	-	-	-	-	-
4+	4+	3+	3+	1+	-	-	-	-	-	-	-
4+	4+	3+	3+	2+	-	-	-	-	-	-	-
4+	4+	1+	3+	1+	-	-	-	-	-	-	-
4+	4+	1+	3+	-	-	-	-	-	-	-	-

Figure 1B. Monolayer - virus + enzyme A

Virus Dilution	Enzyme Dilution											
	-1	-2	-3	-4	-5	-6	-7	-8	-9	-10	-11	-12
-2	3+	3+	4+	4+	1+	4+	4+	4+	4+	4+	4+	4+
-3	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
-4	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
-5	3+	3+	3+	2+	3+	2+	3+	2+	3+	3+	3+	3+
-6	2+	2+	1+	-	-	-	-	1+	-	1+	-	1+
-7	2+	2+	-	-	-	-	-	-	-	-	-	-
-8	2+	1+	-	-	-	-	-	-	-	-	-	-
-9	2+	1+	-	-	-	-	-	-	-	-	-	-

Figure 1C. Monolayer - virus + enzyme B

Virus Dilution	Enzyme Dilution											
	-1	-2	-3	-4	-5	-6	-7	-8	-9	-10	-11	-12
-2	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
-3	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
-4	4+	4+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
-5	4+	3+	3+	2+	2+	3+	3+	3+	3+	3+	3+	3+
-6	4+	4+	3+	-	1+	-	-	-	-	-	-	-
-7	4+	3+	1+	-	-	-	-	-	-	-	-	-
-8	4+	3+	-	-	-	-	-	-	-	-	-	-
-9	4+	3+	-	-	-	-	-	-	-	-	-	-

Figure 1D. Monolayer - virus + enzyme C

Virus Dilution	Enzyme Dilution											
	-1	-2	-3	-4	-5	-6	-7	-8	-9	-10	-11	-12
-2	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
-3	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
-4	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
-5	4+	4+	3+	2+	3+	3+	3+	3+	3+	3+	3+	3+
-6	4+	3	2+	1+	-	1+	1+	1+	-	1+	1+	1+
-7	4+	3+	2+	2+	-	1+	1+	1+	-	-	-	-
-8	4+	3+	2+	1+	-	1+	-	-	-	-	-	-
-9	4+	3+	3+	-	-	-	-	-	-	-	-	-

Figure 1E. Monolayer - virus + enzyme D

Virus Dilution	Enzyme Dilution											
	-1	-2	-3	-4	-5	-6	-7	-8	-9	-10	-11	-12
-2	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
-3	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
-4	4+	4+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
-5	4+	4+	3+	3+	2+	3+	3+	4+	3+	3+	3+	3+
-6	4+	3+	3+	1+	2+	2+	2+	1+	3+	1+	2+	2+
-7	4+	3+	3+	1+	1+	1+	1+	1+	1+	-	-1+	-
-8	4+	3+	2+	-	-	-	-	-	1+	-	-	-
-9	4+	3+	3+	-	-	-	-	-	-	-	-	-



Figure 2A. Suspension - control

Virus Dilution	Enzyme Dilution											
	-1	-2	-3	-4	-5	-6	-7	-8	-9	-10	-11	-12
-2	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
-3	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
-4	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
-5	3+	3+	3+	3+	3+	2+	2+	2+	3+	3+	3+	3+
-6	3+	3+	3+	3+	2+	2+	3+	3+	1+	1+	2+	2+
-7	3+	3+	3+	-	-	-	1+	-	-	-	-	-
-8	2+	2+	2+	-	-	-	-	-	2+	-	-	-
-9	2+	2+	2+	-	-	-	-	-	-	-	-	-

Figure 2B. Suspension - virus + enzyme A

Virus Dilution	Enzyme Dilution											
	-1	-2	-3	-4	-5	-6	-7	-8	-9	-10	-11	-12
-2	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
-3	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
-4	4+	3+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
-5	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+
-6	2+	2+	2+	2+	1+	1+	1+	1+	-	-	-	-
-7	2+	1+	-	-	-	-	-	-	-	-	-	-
-8	1+	-	-	-	-	-	-	-	-	-	-	-
-9	2+	1+	-	-	-	-	-	-	-	-	-	-

Figure 2C. Suspension - virus + enzyme B

Virus Dilution	Enzyme Dilution											
	-1	-2	-3	-4	-5	-6	-7	-8	-9	-10	-11	-12
-2	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+		
-3	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+		
-4	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+		
-5	4+	4+	4+	1+	2+	2+	2+	3+	2+	2+	2+	2+
-6	4+	4+	4+	2+	2+	2+	2+	1+	-	-	1+	-
-7	4+	4+	4+	-	-	-	1+	-	-	-	-	-
-8	4+	4+	4+	-	-	1+	-	-	1+	-	-	-
-9	4+	4+	4+	-	-	-	-	-	-	-	-	-

Figure 2D. Suspension - virus + enzyme C

Virus Dilution	Enzyme Dilution											
	-1	-2	-3	-4	-5	-6	-7	-8	-9	-10	-11	-12
-2	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
-3	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
-4	4+	4+	4+	3+	3+	3+	3+	3+	3+	3+	3+	
-5	4+	4+	3+	3+	2+	2+	2+	1+	3+	3+	2+	
-6	4+	4+	1+	1+	1+	2+	2+	1+	1+	-	2+	
-7	4+	4+	-	-	-	-	1+	-	-	-	-	
-8	4+	4+	-	1+	-	-	2+	-	1+	-	-	
-9	4+	4+	3+	-	-	-	-	-	-	-	-	

Figure 2E. Suspension - virus + enzyme D

Virus Dilution	Enzyme Dilution											
	-1	-2	-3	-4	-5	-6	-7	-8	-9	-10	-11	-12
-2	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
-3	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
-4	4+	4+	4+	3+	3+	3+	3+	3+	3+	3+	3+	3+
-5	4+	4+	4+	2+	3+	2+	1+	1+	1+	1+	2+	2+
-6	4+	4+	4+	1+	-	2+	2+	2+	2+	-	-	2+
-7	4+	4+	3+	-	-	-	1+	-	1+	-	-	-
-8	4+	4+	3+	-	-	-	-	-	-	-	-	-
-9	4+	4+	3+	-	-	-	-	-	-	-	-	-

- 25 -

CLAIMS

1. A composition for the antiviral treatment of a  
5 human or animal, comprising an enzyme as an active  
antiviral agent.
2. A composition as claimed in Claim 1, further  
comprising a synergist for said enzyme.
- 10 3. A composition as claimed in Claim 1 or 2,  
comprising a proteinase.
4. A composition as claimed in any of Claims 1, 2 or  
15 3, comprising a hydroxylase.
5. A composition for the antiviral treatment of a  
human or animal, the composition containing one or  
more active antiviral agent(s) based on or  
20 extracted from the saliva of a blood-feeding  
arthropod, or replicating in whole or in part one  
or more component(s) of such saliva.
6. A composition as claimed in Claim 5, wherein the  
25 arthropod is an insect of the family  
Ceratopogonidae (midges) or of the family  
Culicidae (mosquitos).
7. A composition as claimed in any of Claims 1 to 4  
30 and as claimed in Claims 5 or 6.
8. A composition as claimed in any preceding claim,  
for the prophylactic treatment of a human or  
animal.

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9. A composition as claimed in any preceding claim, for the treatment of a human against a piconavirus, a togavirus or a retrovirus.
- 5 10. A composition as claimed in any preceding claims, for the prophylactic treatment of a human, the composition being adapted for application to an epidermal or mucous surface of the human body.
- 10 11. A composition adapted for application to substantially the entire epidermal surface of the human body, the composition comprising an active antiviral agent and being such that after such application the antiviral agent is present on  
15 substantially the entire epidermal surface of the human body for a substantial period.
12. A composition as claimed in Claim 11, the composition being an emulsion which can be applied  
20 to the human body by an aqueous delivery means.
13. A composition as claimed in Claim 11 or 12 further comprising one or more ingredients selected from the following: antimicrobial agents (including  
25 antibacterial and antifungal agents); insect repellents; sun screening agents; perfumes; deodorants; antiperspirants; humectants; essential oils.
- 30 14. A method for antiviral treatment, the method comprising the application or administration of a composition as claimed in any preceding claim.

## INTERNATIONAL SEARCH REPORT

International Application No  
PCT/GB 96/01968

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K38/43 A61K35/64 A61K9/00 A61K7/40

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FR,M,4 248 (SOCIÉTÉ PLURIPHARM) 1 August 1966	1,2,4, 7-9,14
Y	see the whole document	5,6
X	WO,A,89 11294 (NIKA HEALTH CARE, INC.) 30 November 1989	1,4,7-14
Y	see page 7, line 12 - line 23; claims; examples 2,11-13	5,6
	-/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

## \* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance  
 "E" earlier document but published on or after the international filing date  
 "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)  
 "O" document referring to an oral disclosure, use, exhibition or other means  
 "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention  
 "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone  
 "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.  
 "&" document member of the same patent family

Date of the actual completion of the international search

29 November 1996

Date of mailing of the international search report

20.12.96

Name and mailing address of the ISA

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Authorized officer

Ryckebosch, A

## INTERNATIONAL SEARCH REPORT

 Int ional Application No  
 PCT/GB 96/01968

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CHEMICAL ABSTRACTS, vol. 118, no. 23, 7 June 1993 Columbus, Ohio, US; abstract no. 230457p, G.E. PIMENTAL: "A LYSOZYME-LIKE PROTEIN IN THE SALIVARY GLANDS OF ADULT AEDES AEGYPTI: FUNCTIONAL AND BIOCHEMICAL CHARACTERISTICS." page 630; XP002019869 see abstract & DISS. ABSTR. INT. B, vol. 52, no. 12, PT. 1, 1992, pages 6198-6199, ---	5,6
X	EP,A,0 421 022 (MUCOS EMULSIONSGESELLSCHAFT M.B.H.) 10 April 1991 see the whole document ---	1,3,4, 7-9,14
A	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 92, January 1995, WASHINGTON US, pages 694-698, XP002019867 D.E. CHAMPAGNE ET AL.: "THE SALIVARY GLAND-SPECIFIC APYRASE OF THE MOSQUITO AEDES AEGYPTI IS A MEMBER OF THE 5'-NUCLEOTIDASE FAMILY." cited in the application see the whole document ---	5,6
A	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 91, January 1994, WASHINGTON US, pages 138-142, XP002019868 D.E. CHAMPAGNE ET AL. : "SIALOKININ I AND II: VASODILATORY TACHYKININS FROM THE YELLOW FEVER MOSQUITO AEDES AEGYPTI." see the whole document ---	5,6
A	CHEMICAL ABSTRACTS, vol. 122, no. 15, 10 April 1995 Columbus, Ohio, US; abstract no. 183662x, M.G. NOVAK ET AL.: "5-HYDROXYTRYPTAMINE IN THE SALIVARY GLANDS OF ADULT FEMALE AEDES AEGYPTI AND ITS ROLE IN REGULATION OF SALIVATION." page 657; XP002019870 see abstract & J. EXP. BIOL., vol. 198, no. 1, 1995, pages 167-174, -----	5,6

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB 96/ 01968

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 14  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claim 14 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 96/01968

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
FR-M-4248		NONE	
WO-A-8911294	30-11-89	AU-B- 624331	11-06-92
		AU-A- 1940888	12-12-89
		BG-B- 60544	28-08-95
		CA-A- 1335425	02-05-95
		EP-A- 0380484	08-08-90
		HK-A- 42394	13-05-94
		IL-A- 90092	30-03-95
		JP-B- 6049659	29-06-94
		JP-T- 3504121	12-09-91
		SG-A- 24694	10-06-94
		US-A- 5200182	06-04-93
		US-A- 5466449	14-11-95
EP-A-421022	10-04-91	NONE	